

Patent
Attorney's Docket No. 024916-010

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
Pamela A. SOKOL et al.) Group Art Unit: Unassigned
Application No.: Divisional of Application) Examiner: Unassigned
 Serial No. 09/275,417)
Filed: November 16, 2001)
For: METHOD OF PROTECTION)
 AGAINST ZINC)
 METALLOPROTEASE-SECRETING)
 PATHOGENS)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Kindly insert the attached paper copy of the Sequence Listing between the last page of the Disclosure (page 38) and the first page of the claims, and renumber the pages accordingly.

Please insert the attached Abstract, on a separate sheet, after the last page of the claims (currently page 43).

Kindly replace the paragraph beginning at page 3, line 31, with the following:

-- The inventors have identified two regions of the *P. aeruginosa* elastase amino acid sequence, and within these regions, two epitopes, which are recognized by antibodies which neutralize the proteolytic activity of *P. aeruginosa* elastase and other thermolysin-like proteases.--

Kindly replace the paragraph beginning at page 4, line 14, with the following:

--In accordance with one embodiment, the invention provides a peptide comprising the amino acid sequence VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID NO:1) or a fragment or analogue thereof.--

Kindly replace the paragraph beginning at page 4, line 18, with the following:

--In accordance with a further embodiment, the invention provides a peptide comprising the amino acid sequence HGFTEQNSG (Sequence ID NO:2).--

Kindly replace the paragraph beginning at page 4, line 21, with the following:

--In accordance with a further embodiment, the invention provides a peptide comprising the amino sequence SGALRYMDQPSRDGRSIDM (Sequence ID NO:11).--

Kindly replace the paragraph beginning at page 4, line 26, with the following:

--In accordance with a further embodiment, the invention provides a peptide comprising the amino acid sequence RYMDQPSRD (Sequence ID NO:14).--

Kindly replace the paragraph beginning at page 4, line 29, with the following:

--In accordance with a further embodiment, the invention provides an immunogenic composition comprising at least one active component selected from the group consisting of:

- (a) a peptide comprising the amino acid sequence HGFTEQNSG
(Sequence ID NO:3);
- (b) a peptide comprising the amino acid sequence RYMDQPSRD
(Sequence ID NO:14);
- (c) a peptide comprising the amino acid sequence
VSHGFTEQNSGLIYRGQQSGGMNEAF (Sequence ID
NO:1);
- (d) a peptide comprising the amino acid sequence
SGALRYMDQPSRDGRSIDM (Sequence ID NO:11);
- (e) A fragment or analogue of a peptide of (a), (b), (c) or (d);
- (f) a purified and isolated nucleic acid molecule encoding a
peptide of (a), (b), (c) or (d); and
- (g) a nucleotide sequence which hybridises under stringent
conditions to any of the nucleic acid molecules of (f)

and a pharmaceutically acceptable carrier, the at least one active component producing an immune response when administered to a host.

Kindly replace the paragraph beginning at page 5, line 13, with the following:

--In accordance with a further embodiment, the invention provides an antibody or antiserum specific for a peptide selected from the group consisting of

- (a) VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID NO:1);
- (b) SGALRYMDQPSRDGRSIDM (Sequence ID NO:11);
- (c) HGFTEQNSG (Sequence ID NO:3);
- (d) RYMDQPSRD (Sequence ID NO:14); and
- (e) a fragment or analogue of a peptide of (a), (b), (c) or (d).--

Kindly replace the paragraph beginning at page 5, line 26, with the following:

--In accordance with a further embodiment, the invention provides a purified isolated nucleic acid molecule encoding a peptide selected from the group consisting of

- (a) VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID NO:1);
- (b) SGALRYMDQPSRDGRSIDM (Sequence ID NO:11);
- (c) HGFTEQNSG (Sequence ID NO:3);
- (d) RYMDQPSRD (Sequence ID NO:14); and
- (e) a fragment or analogue of a peptide of (a), (b), (c) or (d).--

Kindly replace the paragraph beginning at page 5, line 36, with the following:

--In accordance with a further embodiment, the invention provides a method of producing a vaccine comprising administering an immunogenic composition comprising at least one active component selected from the group consisting of

- (a) a peptide comprising the amino acid sequence HGFTEQNSG
(Sequence ID NO:3);
- (b) a peptide comprising the amino acid sequence RYMDQPSRD
(Sequence ID NO:14);
- (c) a peptide comprising the amino acid sequence
VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID
NO:1);
- (d) a peptide comprising the amino acid sequence
SGALRYMDQPSRDGRSIDM (Sequence ID NO:11);
- (e) a fragment or analogue of a peptide of (a), (b), (c) or (d);
- (f) a purified and isolated nucleic acid molecule encoding a
peptide of (a), (b), (c) or (d); and
- (g) a nucleotide sequence which hybridises under stringent
conditions to any of the nucleic acid molecules of (f)

and a pharmaceutically acceptable carrier to a test host to determine an amount and a frequency of administration of the active component to confer protection against a disease caused by a bacterial pathogen which secretes a zinc metalloprotease, and formulating the active component in a form suitable for administration to a host to be treated in accordance with the determined amount and frequency of administration.--

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Kindly insert the following paragraph on page 7, after line 6:

-- HGFTEQNSG = Sequence ID NO: 3; RYMDQPSRD = Sequence ID NO. 14;
RYFDQPSRD = Sequence ID NO. 20; HAVTDYTAG = Sequence NO. 21;
RSMSDPAKY = Sequence NO. 22; NGGVHINSG = Sequence NO. 23; VYTPGISGD =
Sequence NO. 24; SYWEEQNTG = Sequence NO. 25; AYSSAPLLD = Sequence NO.
26; ITFTEVAAG = Sequence NO. 27; LYGANPSTR = Sequence NO. 28;
HYAAAPLLD = Sequence NO. 29.--

Kindly replace the paragraph beginning at page 8, line 28, with the following:

--All antibodies examined reacted strongly with peptides from two stretches of the
P. aeruginosa elastase amino acid sequence, amino acids
₃₃₉VSHGFTEQNSGLIYRGQSGGMNEAF₃₆₃ (Sequence ID NO:1) and
₃₉₁SGALRYMDQPSRDGRSIDM₄₀₉ (Sequence ID NO:11). Table 1 shows the overlapping
9 mer peptides within each of these stretches which reacted with the antibodies, namely
peptides 14 to 22 for amino acids 339 to 363 and peptides 40-45 for amino acids 391 to
409. Figure 1 shows the epitope mapping results.--

Kindly replace the paragraph beginning at page 9, line 1, with the following:

--All of the antibodies reacted most strongly with peptide 15 (₃₄₁HGFTEQNSG₃₄₉)
(Sequence ID NO:3) and peptide 42 (₃₉₅RYMDQPSRD₄₀₃) (Sequence ID NO:14).

Kindly replace the paragraph beginning at page 9, line 4, with the following:

--Peptide 15 overlaps the ₃₃₇HExxH₃₄₁ active site found in elastase. Antibody binding to the amino acid sequence ₃₄₁HGFTEQNSG₃₄₉ (Sequence ID NO:13) would explain the ability of these antibodies to neutralize elastase. An identical sequence is found in *V. cholerae* HA/protease (Fig. 2). Three of nine residues match the thermolysin sequence in the region of the HExxH motif, which may be sufficient for antibody binding and neutralization. A better match is found, however, in the region spanning residues 227-235, with four of nine residues identical to peptide 15. This region spans the Histidine at residue 231, which acts as a proton donor at the active site. Antibody binding to this epitope may be the reason that thermolysin is inactivated.

Kindly replace the paragraph beginning at page 9, line 18, with the following:

--Peptide 42 (₃₉₅RYMDQPSRD₄₀₃) (Sequence ID NO:14) is located between E₃₆₁, which binds a zinc atom, and H₄₂₀, which acts as a proton donor at the active site (8, 30). The binding of antibodies to this epitope could effectively inhibit proteolytic activity by directly blocking the active site cleft. A nearly identical sequence is present in *V. cholerae* HA/protease, with eight of nine residues identical (Fig. 2). There is less homology between elastase and thermolysin in this region. Two possible epitopes recognized by the antibodies are between residues 192-200 and residues 203-211 of elastase. Both epitopes match peptide 42 with three of nine residues identical. Both sequences are located between the zinc binding site and the H₂₃₁ which serves as the proton donor.

Kindly replace the paragraph beginning at page 12, line 33, with the following:

--Immunogenic compositions, suitable to be used as vaccines, may be prepared from peptide VSHGFTEQNSGLIYRGQSGMNEAF (Sequence ID NO:1), peptide SGALRYMDQPSRDGRSIDM (Sequence ID NO:11) and fragments and analogues thereof, as disclosed herein.

Kindly replace the paragraph beginning at page 12, line 37, with the following:

--Peptides of about nine consecutive amino acids selected from the sequences VSHGFTEQNSGLIYRGQSGMNEAF (Sequence ID NO:1) and SGALRYMDQPSRDGRSIDM (Sequence ID NO:11), or analogues of such peptides, are preferred.

Kindly replace the paragraph beginning at page 13, line 4, with the following:

--Peptides HGFTEQNSG (Sequence ID NO:3) and RYMDQPSRD (Sequence ID NO:14) are especially preferred. An immunogenic composition may also be prepared from a peptide mixture, such a mixture of peptides HGFTEQNSG (Sequence ID NO:3) and RYMDQPSRD (Sequence ID NO:14).

Kindly replace the paragraph beginning at page 20, line 28, with the following:

--In order to prepare peptides for production of polyclonal antibodies, fusion proteins containing a selected peptide, such as peptide 15 or peptide 42, can be synthesized in bacteria by expression of corresponding DNA sequences in a suitable cloning vehicle.

Fusion proteins are commonly used as a source of antigen for producing antibodies. Two widely used expression systems for *E. coli* are lacZ fusions using the pUR series of vectors and trpE fusions using the pATH vectors. The peptides can then be purified, coupled to a carrier protein if desired, and mixed with Freund's adjuvant (to help stimulate the antigenic response of the animal) and injected into rabbits or other appropriate laboratory animals.--

Kindly replace the paragraph beginning at page 24, line 14, with the following:

--**Polyclonal antibody production.** Peptides HGFTEQNSG (Sequence ID NO:3) (peptide 15) and RYMDQPSRF (Sequence ID NO:14) (PEPTIDE 42) AND THE SAME PEPTIDES CONJUGATED TO BOVINE SERUM ALBUMIN (BSA) or keyhole limpet hemocyanin (KLH) were synthesized by the Alberta Peptide Institute, Edmonton, Canada. Conjugates were prepared for immunization as suggested by the manufacturer. The BSA conjugates were prepared in complete Freund's adjuvant and 0.5 ml containing 0.1 mg of either peptide 15-BSA or peptide 42-BSA was injected in each thigh of two New Zealand white rabbits. Rabbits immunized with peptide 42-BSA were boosted with intramuscular injections of peptide in incomplete Freund's three times at bi-weekly intervals. Rabbits immunized with peptide 15-BSA were given two additional boosts as test bleeds indicated lower titres.

Kindly replace the paragraph beginning at page 26, line 26, with the following:

--**Epitope mapping of the 13.9 kDa NCS-elastase fragment.** Sixty overlapping 9-mer peptides, with a two amino acid offset, were synthesized spanning the 13.9 kDa NCS-

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elastase fragment. The first peptide was DGTAMLFGD (Sequence ID NO:18) and the last was YLLANSPGW (Sequence ID NO:19). In addition, two control peptides were synthesized, PLRQ (positive) and GLAQ (negative). Essentially the same results were found with all five antibodies. The results with two representative antibodies, Mabs 36-6-6 and 36-6-8, are shown in Figure 1. MAbs 36-6-6 and 36-6-8 bound most strongly to peptides 15 (₃₄₁HGFTEQNSG₃₄₉) (Sequence ID NO:3) and 42 (₃₉₅RYMDQPSRD₄₀₃) (Sequence ID NO:14). Numbering of the amino acids is from the elastase precursor. Peptides with sequences overlapping these peptides were also recognized to a lesser degree. Two control peptide pins were provided with the kit and these peptides responded as expected with the control antibody provided (see Fig. 1).--

Kindly replace the paragraph beginning at page 29, line 34, with the following:

--Rats were infected intratracheally using the agar bead model of Cash et al. (41) with a wild type strain of *P. aeruginosa* (PAO). At three and seven days after infection, the lungs of five rats in each group were lavaged with BSA. An aliquot of lavage was used to determine the PNM differential count and the remainder frozen at -70°C. On day seven, quantitative bacteriology (41) was performed on five rats from each group and quantitative pathology (42) on 5-7 rats from each group.

Kindly replace Table I on page 35 with the following table:

TABLE 1

P. aeruginosa elastase peptides reacting with MAbs

14	VSHGFTEQN	(Sequence ID NO:2)
15	HGFTEQNSG	(Sequence ID NO:3)
16	FTEQNSGLI	(Sequence ID NO:4)
17	EQNSGLIYR	(Sequence ID NO:5)
18	NSGLIYRGQ	(Sequence ID NO:6)
19	GLIYRGQSG	(Sequence ID NO:7)
20	IYRGQSGGM	(Sequence ID NO:8)
21	RQQSGGMNE	(Sequence ID NO:9)
22	QSGGMNEAF	(Sequence ID NO:10)
40	SGALRYMDQ	(Sequence ID NO:12)
41	ALRYMDQPS	(Sequence ID NO:13)
42	RYMDQPSRD	(Sequence ID NO:14)
43	MDQPSRDGR	(Sequence ID NO:15)
44	QPSRDGRSI	(Sequence ID NO:16)
45	SRDGRSIDM	(Sequence ID NO:17)

IN THE CLAIMS:

Please cancel claim 1 without prejudice or disclaimer to the subject matter disclosed therein.

Kindly add new claims 25 and 26 as follows:

--25. An antibody or antiserum specific for a peptide selected from the group consisting of

- (a) VSHGFTEQNSGLIYRGQSGGMNEAF;
- (b) SGALRYMDQPSRDGRSIDM;
- (c) HGFTEQNSG;
- (d) RYMDQPSRD; and
- (e) a fragment or analogue of a peptide of (a), (b), (c) or (d).

26. A method of producing a vaccine comprising administering an immunogenic composition comprising at least one active component selected from the group consisting of:

- (a) a peptide comprising the amino acid sequence HGFTEQNSG;
- (b) a peptide comprising the amino acid sequence RYMDQPSRD;
- (c) a peptide comprising the amino acid sequence
VSHGFTEQNSGLIYRGQSGGMNEAF;
- (d) a peptide comprising the amino acid sequence
SGALRYMDQPSRDGRSIDM;
- (e) a fragment or analogue of a peptide of (a), (b), (c) or (d);
- (f) a purified and isolated nucleic acid molecule encoding a peptide of (a), (b),
(c) or (d); and

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(g) a nucleotide sequence which hybridizes under stringent conditions to any of the nucleic acid molecules of (f)

and a pharmaceutically acceptable carrier, to a test host to determine an amount and a frequency of administration of the active component to confer protection against a disease caused by a bacterial pathogen which secretes a zinc metalloprotease, and formulating the active component in a form suitable for administration to a host to be treated in accordance with said determined amount and frequency of administration.

U.S. GOVERNMENT PRINTING OFFICE: 1997 500-100-000

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REMARKS

Entry of the foregoing and prompt and favorable consideration of the subject application are respectfully requested.

By the present amendment, a paper copy of the Sequence Listing for the subject application has been added between the last page of the specification currently page 38, and the first page of the claims, currently page 39. Please renumber the page numbers accordingly. An Abstract on a separate sheet of paper, has also been added after the last page of the claims, currently page 43. Further, the specification has been amended in order to conform to the amendments made to the specification in the parent application including identifying the appropriate sequence identifiers throughout the application and correcting several grammatical and/or typographical errors. Moreover, by the foregoing, claim 1 has been canceled without prejudice or disclaimer to the subject matter disclosed therein and claims 25 and 26 have been added. Support for these claims can be found throughout the entire application as originally filed. No new matter has been added.

In the event that there are any questions relating to this Preliminary Amendment, or the application in general, it would be appreciated if the Examiner would telephone the

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undersigned attorney concerning such questions so that prosecution of this application may
be expedited.

Respectfully submitted,

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Date: November 16, 2001

U.S. PATENT AND TRADEMARK OFFICE

Attachment to Preliminary Amendment dated November 16, 2001

Marked-up Copy

Page 3, Paragraph Beginning at Line 31

The inventors have identified two regions of the *P. aeruginosa* elastase amino acid sequence, and within these regions, two tptopes, which are recognized by antibodies which [neutralise] neutralize the proteolytic activity of *P. aeruginosa* elastase and other thermolysin-like proteases.

Page 4, Paragraph Beginning at Line 14

In accordance with one embodiment, the invention provides a peptide comprising the amino acid sequence VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID NO:1) or a fragment or analogue thereof.

Page 4, Paragraph Beginning at Line 18

In accordance with a further embodiment, the invention provides a peptide comprising the amino acid sequence HGFTEQNSG (Sequence ID NO:2).

Page 4, Paragraph Beginning at Line 21

In accordance with a further embodiment, the invention provides a peptide comprising the amino sequence SGALRYMDQPSRDGRSIDM (Sequence ID NO:11).

Page 4, Paragraph Beginning at Line 26

In accordance with a further embodiment, the invention provides a peptide comprising the amino acid sequence RYMDQPSRD (Sequence ID NO:14).

Page 4, Paragraph Beginning at Line 29

--In accordance with a further embodiment, the invention provides an immunogenic composition comprising at least one active component selected from the group consisting of:

- (a) a peptide comprising the amino acid sequence HGFTEQNSG (Sequence ID NO:3);
- (b) a peptide comprising the amino acid sequence RYMDQPSRD (Sequence ID NO:14);
- (c) a peptide comprising the amino acid sequence VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID NO:1);
- (d) a peptide comprising the amino acid sequence SGALRYMDQPSRDGRSIDM (Sequence ID NO:11);
- (e) A fragment or analogue of a peptide of (a), (b), (c) or (d);
- (f) a purified and isolated nucleic acid molecule encoding a peptide of (a), (b), (c) or (d); and
- (g) a nucleotide sequence which hybridises under stringent conditions to any of the nucleic acid molecules of (f)

and a pharmaceutically acceptable carrier, the at least one active component producing an immune response when administered to a host.

Page 5, Paragraph Beginning at Line 13

--In accordance with a further embodiment, the invention provides an antibody or antiserum specific for a peptide selected from the group consisting of

- (a) VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID NO:1);
- (b) SGALRYMDQPSRDGRSIDM (Sequence ID NO:11);
- (c) HGFTEQNSG (Sequence ID NO:3);
- (d) RYMDQPSRD (Sequence ID NO:14); and
- (e) a fragment or analogue of a peptide of (a), (b), (c) or (d).--

Page 5, Paragraph Beginning at Line 26

--In accordance with a further embodiment, the invention provides a purified isolated nucleic acid molecule encoding a peptide selected from the group consisting of

- (a) VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID NO:1);
- (b) SGALRYMDQPSRDGRSIDM (Sequence ID NO:11);
- (c) HGFTEQNSG (Sequence ID NO:3);
- (d) RYMDQPSRD (Sequence ID NO:14); and
- (e) a fragment or analogue of a peptide of (a), (b), (c) or (d).--

Page 5, Paragraph Beginning at Line 36

--In accordance with a further embodiment, the invention provides a method of producing a vaccine comprising administering an immunogenic composition comprising at least one active component selected from the group consisting of

- (a) a peptide comprising the amino acid sequence HGFTEQNSG
(Sequence ID NO:3);
- (b) a peptide comprising the amino acid sequence RYMDQPSRD
(Sequence ID NO:14);
- (c) a peptide comprising the amino acid sequence
VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID
NO:1);
- (d) a peptide comprising the amino acid sequence
SGALRYMDQPSRDGRSIDM (Sequence ID NO:11);
- (e) a fragment or analogue of a peptide of (a), (b), (c) or (d);
- (f) a purified and isolated nucleic acid molecule encoding a peptide of (a), (b), (c) or (d); and
- (g) a nucleotide sequence which hybridises under stringent conditions to any of the nucleic acid molecules of (f)

and a pharmaceutically acceptable carrier to a test host to determine an amount and a frequency of administration of the active component to confer protection against a disease caused by a bacterial pathogen which secretes a zinc metalloprotease, and formulating the

active component in a form suitable for administration to a host to be treated in accordance with the determined amount and frequency of administration.--

Page 8, Paragraph Beginning at Line 28

--All antibodies examined reacted strongly with peptides from two stretches of the *P. aeruginosa* elastase amino acid sequence, amino acids ₃₃₉VSHGFTEQNSGLIYRGQSGGMNEAF₃₆₃ (Sequence ID NO:1) and ₃₉₁SGALRYMDQPSRDGRSIDM₄₀₉ (Sequence ID NO:11). Table 1 shows the overlapping 9 mer peptides within each of these stretches which reacted with the antibodies, namely peptides 14 to 22 for amino acids 339 to 363 and peptides 40-45 for amino acids 391 to 409. Figure 1 shows the epitope mapping results.

Page 9, Paragraph Beginning at Line 1

All of the antibodies reacted most strongly with peptide 15 (₃₄₁HGFTEQNSG₃₄₉) (Sequence ID NO:3) and peptide 42 (₃₉₅RYMDQPSRD₄₀₃) (Sequence ID NO:14).

Page 9, Paragraph Beginning at Line 4

Peptide 15 overlaps the ₃₃₇HExxH₃₄₁ active site found in elastase. Antibody binding to the amino acid sequence ₃₄₁HGFTEQNSG₃₄₉ (Sequence ID NO:13) would explain the ability of these antibodies to neutralize elastase. An identical sequence is found in *V. cholerae* HA/protease (Fig. 2). Three of nine residues match the thermolysin sequence in the region of the HExxH motif, which may be sufficient for antibody binding and

neutralization. A better match is found, however, in the region spanning residues 227-235, with four of nine residues identical to peptide 15. This region spans the Histidine at residue 231, which acts as a proton donor at the active site. Antibody binding to this epitope may be the reason that thermolysin is inactivated.

Page 9, Paragraph Beginning at Line 18

Peptide 42 (₃₉₅RYMDQPSRD₄₀₃) (Sequence ID NO:14) is located between E₃₆₁, which binds a zinc atom, and H₄₂₀, which acts as a proton donor at the active site (8, 30). The binding of antibodies to this epitope could effectively inhibit proteolytic activity by directly blocking the active site cleft. A nearly identical sequence is present in *V. cholerae* HA/protease, with eight of nine residues identical (Fig. 2). There is less homology between elastase and thermolysin in this region. Two possible epitopes recognized by the antibodies are between residues 192-200 and residues 203-211 of elastase. Both epitopes match peptide 42 with three of nine residues identical. Both sequences are located between the zinc binding site and the H₂₃₁ which serves as the proton donor.

Page 12, Paragraph Beginning at Line 33

Immunogenic compositions, suitable to be used as vaccines, may be prepared from peptide VSHGFTEQNSGLIYRGQSGMNEAF (Sequence ID NO:1), peptide SGALRYMDQPSRDGRSIDM (Sequence ID NO:11) and fragments and analogues thereof, as disclosed herein.

Page 12, Paragraph Beginning at Line 37

Peptides of about nine consecutive amino acids selected from the sequences

VSHGFTEQNSGLIYRGQSGMNEAF (Sequence ID NO:1) and
SGALRYMDQPSRDGRSIDM (Sequence ID NO:11), or analogues of such peptides, are
preferred.

Page 13, Paragraph Beginning at Line 4

Peptides HGFTEQNSG (Sequence ID NO:3) and RYMDQPSRD (Sequence ID
NO:14) are especially preferred. An immunogenic composition may also be prepared from
a peptide mixture, such a mixture of peptides HGFTEQNSG (Sequence ID NO:3) and
RYMDQPSRD (Sequence ID NO:14).

Page 20, Paragraph Beginning at Line 28

In order to prepare peptides for production of polyclonal antibodies, fusion proteins
containing a selected [peptide] peptide, such as peptide 15 or peptide 42, can be
synthesized in bacteria by expression of corresponding DNA sequences in a suitable
cloning vehicle. Fusion proteins are commonly used as a source of antigen for producing
antibodies. Two widely used expression systems for *E. coli* are lacZ fusions using the
pUR series of vectors and trpE fusions using the pATH vectors. The peptides can then be
purified, coupled to a carrier protein if desired, and mixed with Freund's adjuvant (to help
stimulate the antigenic response of the animal) and injected into rabbits or other appropriate
laboratory animals.

Page 24, Paragraph Beginning at Line 14

Polyclonal antibody production. Peptides HGFTEQNSG (Sequence ID NO:3) (peptide 15) and RYMDQPSRF (Sequence ID NO:14) (PEPTIDE 42) and the same peptides conjugated to bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH) were synthesized by the Alberta Peptide Institute, Edmonton, Canada. Conjugates were prepared for immunization as suggested by the manufacturer. The BSA conjugates were prepared in complete Freund's adjuvant and 0.5 ml containing 0.1 mg of either peptide 15-BSA or peptide 42-BSA was injected in each thigh of two New Zealand white rabbits. Rabbits immunized with peptide 42-BSA were boosted with intramuscular injections of peptide in incomplete Freund's three times at bi-weekly intervals. Rabbits immunized with peptide 15-BSA were given two additional boosts as test bleeds indicated lower titres.

Page 26, Paragraph Beginning at Line 26

Epitope mapping of the 13.9 kDa NCS-elastase fragment. Sixty overlapping 9-mer peptides, with a two amino acid offset, were synthesized spanning the 13.9 kDa NCS-elastase fragment. The first peptide was DGTAMLFGD (Sequence ID NO:18) and the last was YLLANSPGW (Sequence ID NO:19). In addition, two control peptides were synthesized, PLRQ (positive) and GLAQ (negative). Essentially the same results were found with all five antibodies. The results with two representative antibodies, Mabs 36-6-6 and 36-6-8, are shown in Figure 1. MAbs 36-6-6 and 36-6-8 bound most strongly to peptides 15 (₃₄₁HGFTEQNSG₃₄₉) (Sequence ID NO:3) and 42 (₃₉₅RYMDQPSRD₄₀₃) (Sequence ID NO:14). Numbering of the amino acids is from the elastase precursor.

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Peptides with sequences overlapping these peptides were also recognized to a lesser degree. Two control peptide pins were provided with the kit and these peptides responded as expected with the control antibody provided (see Fig. 1).

Page 29, Paragraph Beginning at Line 34

Rats were infected intratracheally using the agar bead model of Cash et al. (41) with a wild type strain of *P. aeruginosa* (PAO). At three and seven days after infection, the lungs of five rats in each group [with] were lavaged with BSA. An aliquot of lavage was used to determine the PNM differential count and the remainder frozen at -70°C. On day seven, quantitative bacteriology (41) was performed on five rats from each group and quantitative pathology (42) on 5-7 rats from each group.

Page 35, Paragraph Beginning at Line 1

TABLE 1

P. aeruginosa elastase peptides reacting with MAbs

14	VSHGFTEQN	(Sequence ID NO:2)
15	HGFTEQNSG	(Sequence ID NO:3)
16	FTEQNSGLI	(Sequence ID NO:4)
17	EQNSGLIYR	(Sequence ID NO:5)
18	NSGLIYRGQ	(Sequence ID NO:6)
19	GLIYRGQSG	(Sequence ID NO:7)
20	IYRGQSGGM	(Sequence ID NO:8)
21	RGQSGGMNE	(Sequence ID NO:9)
22	QSGGMNEAF	(Sequence ID NO:10)
40	SGALRYMDQ	(Sequence ID NO:12)
41	ALRYMDQPS	(Sequence ID NO:13)
42	RYMDQPSRD	(Sequence ID NO:14)
43	MDQPSRDGR	(Sequence ID NO:15)
44	QPSRDGRSI	(Sequence ID NO:16)
45	SRDGRSIDM	(Sequence ID NO:17)